(FILE 'HOME' ENTERED AT 15:39:19 ON 10 MAR 2003)

FILE 'BIOSIS, MEDLINE, CAPLUS, EMBASE, CANCERLIT' ENTERED AT 15:39:29 ON 10 MAR 2003

L1	533	INTEGRIN LINKED KINASE
L2	13532	WORTMANNIN
L3	5605	LY294002
L4	842	PHOSPHATIDYLINOSITOL 3,4,5 TRIPHOSPHATE
L5	22	L1 AND L2
L6	2	L5 AND L4
L7	11	L1 AND L3
L8	0	L7 AND L4
L9	11	L1 AND L3
L10	0	L9 AND L4
L11	8	DUP REM L5 (14 DUPLICATES REMOVED)
L12	5	DUP REM L7 (6 DUPLICATES REMOVED)
L13	5	DUP REM L9 (6 DUPLICATES REMOVED)

WEST Search History

DATE: Monday, March 10, 2003

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT,PGPB,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ			
L11	L8 and L5	6	L11
L10	L7 and L5	5	L10
L9	L6 and L5	5	L9
L8	L1 and L4	13	L8
L7	L1 and L3	9	L7
L6	L1 and L2	9	L6
L5	phosphatidylinositol 3,4,5 triphosphate	127	L5
L4	phosphatidylinositol 3,4,5	184	L4
L3	LY294002	94	L3
L2	wortmannin	220	L2
L1	integrin linked kinase	45	L1

END OF SEARCH HISTORY

L6 ANSWER 1 OF 2 MEDLINE
ACCESSION NUMBER: 1998409636 MEDLINE
DOCUMENT NUMBER: 98409636 PubMed ID: 9736715
TITLE: Phosphoinositide-3-OH kinase-dependent regulation of glycogen synthase kinase 3 and protein kinase B/AKT by the **integrin-linked kinase**.
AUTHOR: Delcommenne M; Tan C; Gray V; Rue L; Woodgett J; Dedhar S
CORPORATE SOURCE: British Columbia Cancer Agency, Jack Bell Research Centre, 2660 Oak Street, Vancouver, British Columbia V6H 3Z6 Canada.
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1998 Sep 15) 95 (19) 11211-6. Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199810
ENTRY DATE: Entered STN: 19990106
Last Updated on STN: 20021218
Entered Medline: 19981026

AB **Integrin-linked kinase** (ILK) is an ankyrin-repeat containing serine-threonine protein kinase capable of interacting with the cytoplasmic domains of integrin beta1, beta2, and beta3 subunits. Overexpression of ILK in epithelial cells disrupts cell-extracellular matrix as well as cell-cell interactions, suppresses suspension-induced apoptosis (also called Anoikis), and stimulates anchorage-independent cell cycle progression. In addition, ILK induces nuclear translocation of beta-catenin, where the latter associates with a T cell factor/lymphocyte enhancer-binding factor 1 (TCF/LEF-1) to form an activated transcription factor. We now demonstrate that ILK activity is rapidly, but transiently, stimulated upon attachment of cells to fibronectin, as well as by insulin, in a phosphoinositide-3-OH kinase [Pi(3)K]-dependent manner. Furthermore, phosphatidylinositol(3,4,5)trispho sphate specifically stimulates the activity of ILK in vitro, and in addition, membrane targetted constitutively active Pi(3)K activates ILK in vivo. We also demonstrate here that ILK is an upstream effector of the Pi(3)K-dependent regulation of both protein kinase B (PKB/AKT) and glycogen synthase kinase 3 (GSK-3). Specifically, ILK can directly phosphorylate GSK-3 in vitro and when stably, or transiently, overexpressed in cells can inhibit GSK-3 activity, whereas the overexpression of kinase-deficient ILK enhances GSK-3 activity. In addition, kinase-active ILK can phosphorylate PKB/AKT on serine-473, whereas kinase-deficient ILK severely inhibits endogenous phosphorylation of PKB/AKT on serine-473, demonstrating that ILK is involved in agonist stimulated, Pi(3)K-dependent, PKB/AKT activation. ILK is thus a receptor-proximal effector for the Pi(3)K-dependent, extracellular matrix and growth factor mediated, activation of PKB/AKT, and inhibition of GSK-3.

L6 ANSWER 2 OF 2 CANCERLIT
ACCESSION NUMBER: 1998409636 CANCERLIT
DOCUMENT NUMBER: 98409636 PubMed ID: 9736715
TITLE: Phosphoinositide-3-OH kinase-dependent regulation of glycogen synthase kinase 3 and protein kinase B/AKT by the **integrin-linked kinase**.
AUTHOR: Delcommenne M; Tan C; Gray V; Rue L; Woodgett J; Dedhar S
CORPORATE SOURCE: British Columbia Cancer Agency, Jack Bell Research Centre, 2660 Oak Street, Vancouver, British Columbia V6H 3Z6 Canada.
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1998 Sep 15) 95 (19) 11211-6. Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States

(1); Zhang, Z. (1); Jabali, M. (1); Costello, P. C. (1); Baybik, J. (1); Yoganathan, N. (1); Leung, D. (1); Dedhar, S.; Sanghera, J. (1)

CORPORATE SOURCE: (1) Kinetek Pharmaceuticals Inc., Vancouver, BC, V6P 6G5 Canada

SOURCE: Inflammation Research, (September, 2001) Vol. 50 , No. Supplement 3, pp. S160. print.
Meeting Info.: 5th World Congress on Inflammation Edinburgh, Scotland September 22-26, 2001
ISSN: 1023-3830.

DOCUMENT TYPE: Conference
LANGUAGE: English
SUMMARY LANGUAGE: English

L8 ANSWER 4 OF 8 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 1
ACCESSION NUMBER: 2001:869529 CAPLUS
DOCUMENT NUMBER: 136:132984
TITLE: Downregulation of **integrin-linked kinase** mRNA expression by nitric oxide in rat glomerular mesangial cells

AUTHOR(S): Beck, Karl-Friedrich; Walpen, Sebastian; Eberhardt, Wolfgang; Pfeilschifter, Josef

CORPORATE SOURCE: Pharmazentrum Frankfurt, Klinikum der Johann Wolfgang Goethe-Universitat, Frankfurt am Main, D-60590, Germany

SOURCE: Life Sciences (2001), 69(25/26), 2945-2955
CODEN: LIFSAK; ISSN: 0024-3205

PUBLISHER: Elsevier Science Inc.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Inflammatory glomerular diseases are accompanied by changes in the expression patterns of growth factors, mediators and matrix-assocd. proteins in mesangial cells and by the prodn. of nitric oxide via the cytokine-inducible form of the nitric oxide synthase. Nitric oxide has been shown to act potently on gene transcription. To identify genes that are differentially expressed by endogenously produced nitric oxide, we forced rat mesangial cells to produce high amts. of nitric oxide by exposure to inflammatory cytokines and compared the mRNA expression patterns of cytokine-stimulated mesangial cells with cells that were addnl. treated with the nitric oxide synthase inhibitor L-NMMA to block endogenous NO synthesis. We used a modification of a low stringency RT-PCR approach designated as RNA arbitrarily-primed polymerase chain reaction (RAP-PCR). In this way, we identified among others the **integrin-linked kinase** (ILK) as an NO-regulated gene. The NO-mediated changes in the mRNA and protein expression patterns of ILK were compared to that of "secreted protein acidic and rich in cysteine" (SPARC), a gene that was identified as NO-regulated in the same set of expts. ILK and SPARC mRNA levels were downregulated by cytokines via endogenously produced nitric oxide in a comparable manner as verified by Northern blot anal. In contrast, cytokine-induced NO prodn. or administration of exogenous NO-donors strongly reduced SPARC protein levels without altering ILK protein content in mesangial cells over a period up to 72 h. Blocking de novo protein synthesis showed a short half-life of SPARC (< 2 h) whereas ILK-protein was stable over a period of 7 h, indicating that NO-mediated redn. of ILK mRNA levels does not influence protein content of ILK in mesangial cells under the time limitations given under cell culture conditions. However, a role for cytokines/NO in ILK-long-term regulation in chronic inflammatory diseases that may influence phenotypic responses such as apoptosis or cell proliferation remains to be elucidated.

REFERENCE COUNT: 9 THERE ARE 9 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 5 OF 8 CAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 2002:946143 CAPLUS
DOCUMENT NUMBER: 138:319
TITLE: **Integrin linked kinase** modulation of leukocyte trafficking
INVENTOR(S): Kojic, Ljiljana; Kalmar, Gabe; Moran, David M.
PATENT ASSIGNEE(S): Kinetek Pharmaceuticals, Inc., Can.
SOURCE: PCT Int. Appl., 22 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002098461	A2	20021212	WO 2002-CA831	20020605
W: AU, CA, CN, JP, MX, NO				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR				
US 2003013640	A1	20030116	US 2002-163385	20020604

PRIORITY APPLN. INFO.: US 2001-296262P P 20010605

AB Methods and compns. are provided to modulate the trafficking of leukocytes to specific extravascular sites. The trafficking of leukocytes is prevented by the administration of ILK-blocking agents; compds. that otherwise prevent the binding of natural ILK ligands to ILK; or compds. that prevent expression of, or signaling through, ILK. Compds. that enhance ILK activity increase the trafficking of leukocytes to targeted sites. The modulation of trafficking is used to regulate immune processes at targeted sites, for example to decrease undesirable inflammatory, or atopic and allergic responses.

L8 ANSWER 6 OF 8 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:946108 CAPLUS
DOCUMENT NUMBER: 138:11409
TITLE: **Integrin linked kinase** modulation of macrophage activation
INVENTOR(S): Kojic, Ljiljana; Logan, Patricia M.; Wheeler, Jeffery J.; Sutton, Kymberley L.
PATENT ASSIGNEE(S): Kinetek Pharmaceuticals, Inc., Can.
SOURCE: PCT Int. Appl., 31 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002098419	A1	20021212	WO 2002-US18128	20020605
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				

PRIORITY APPLN. INFO.: US 2001-296181P P 20010605

AB Methods are provided to specifically modulate the activation of monocytes and/or macrophages. Administration of **integrin linked kinase** (ILK) blocking agents; compds. that otherwise prevent the binding of natural ILK ligands to ILK; or compds. that prevent expression

of, or signaling through ILK exert an anti-inflammatory effect by inhibiting iNOS and COX-2 expression, at the level of transcription by suppressing the activation of NF-kB. The modulation of activation through ILK is used to regulate immune processes at targeted sites, for example to decrease undesirable inflammatory responses.

REFERENCE COUNT: 2 THERE ARE 2 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 7 OF 8 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:814680 CAPLUS
DOCUMENT NUMBER: 137:304771
TITLE: Treatment of inflammatory diseases including psoriasis with **integrin-linked kinase** inhibitors
INVENTOR(S): Dedhar, Shoukat; Hannigan, Greg; Hunt, David W. C.; Tao, Jing-Song; Fazli, Ladan
PATENT ASSIGNEE(S): Can.
SOURCE: U.S. Pat. Appl. Publ., 12 pp., Cont.-in-part of U.S. 6,338,958.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 4
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2002155179	A1	20021024	US 2001-998250	20011130
US 6013782	A	20000111	US 1997-955841	19971021
US 6001622	A	19991214	US 1998-35706	19980305
US 6338958	B1	20020115	US 1999-390425	19990903

PRIORITY APPLN. INFO.:
US 1995-9074P P 19951221
US 1996-752345 B2 19961119
US 1997-955841 A2 19971021
US 1998-35706 A1 19980305
US 1999-390425 A2 19990903

AB Inhibitors of **integrin-linked kinase** (ILK) are used in the treatment of inflammatory disease, including cutaneous inflammatory diseases, such as psoriasis, scleroderma, systemic lupus erythematosus and atopic dermatitis. When zymosan was administered to mice, peritoneal cavity neutrophil nos. increased by approx. 4-fold within 4 h. However, if MC-5 was given orally at 200 mg/kg at the time of zymosan administration cells nos. within the peritoneal cavity were equiv. to those of animals that received a saline control solvent 4 h before. Thus, a compd. with specific in vitro anti-ILK activity can affect the influx of cells into a tissue following the delivery of a strong pro-inflammatory signal in vivo.

L8 ANSWER 8 OF 8 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:595504 CAPLUS
DOCUMENT NUMBER: 137:150270
TITLE: **Integrin-linked kinase** and its use
INVENTOR(S): Dedhar, Shoukat; Hannigan, Greg; Yee, Arthur
PATENT ASSIGNEE(S): Can.
SOURCE: U.S. Pat. Appl. Publ., 34 pp., Cont.-in-part of U. S. Ser. No. 390,425.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 4
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE

US 2002107216	A1	20020808	US 2001-925548		20010808
US 6013782	A	20000111	US 1997-955841		19971021
US 6001622	A	19991214	US 1998-35706		19980305
US 6338958	B1	20020115	US 1999-390425		19990903

PRIORITY APPLN. INFO.:

US 1995-9074P	P	19951221
US 1996-752345	B2	19961119
US 1997-955841	A2	19971021
US 1998-35706	A1	19980305
US 1999-390425	A2	19990903

AB ILK genetic sequences and methods of use are provided. Antisense oligonucleotides complementary to ILK are useful in downregulating expression for therapeutic and investigative purposes.

L8 ANSWER 1 OF 8 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:172536 BIOSIS
DOCUMENT NUMBER: PREV200200172536
TITLE: **Integrin-linked kinase** regulates inducible nitric oxide synthase and cyclooxygenase-2 expression in an NF-kappaB-dependent manner.
AUTHOR(S): Tan, Clara; Mui, Alice; Dedhar, Shoukat (1)
CORPORATE SOURCE: (1) Jack Bell Research Center, 2660 Oak St., Vancouver, BC, V6H 3Z6: Sdedhar@interchange.ubc.ca Canada
SOURCE: Journal of Biological Chemistry, (February, 2002) Vol. 277, No. 5, pp. 3109-3116. http://www.jbc.org/. print.
ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Nitric oxide (NO) and prostaglandins are produced as a result of the stimulation of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2, respectively, in response to cytokines or lipopolysaccharide (LPS). We demonstrate that the activity of **integrin-linked kinase** (ILK) is stimulated by LPS activation in J774 macrophages. Inhibition of ILK activity by dominant-negative ILK or a highly selective small molecule ILK inhibitor, in epithelial cells or LPS-stimulated J774 cells and murine macrophages, resulted in inhibition of iNOS expression and NO synthesis. LPS stimulates the phosphorylation of IkappaB on Ser-32 and promotes its degradation. Inhibition of ILK suppressed this LPS-stimulated IkappaB phosphorylation and degradation. Similarly, ILK inhibition suppressed the LPS-stimulated iNOS promoter activity. Mutation of the NF-kappaB sites in the iNOS promoter abolished LPS- and ILK-mediated regulation of iNOS promoter activity. Overexpression of ILK-stimulated NF-kappaB activity and inhibition of ILK or protein kinase B (PKB/Akt) suppressed this activation. We conclude that ILK can regulate NO production in macrophages by regulating iNOS expression through a pathway involving PKB/Akt and NF-kappaB. Furthermore, we also demonstrate that ILK activity is required for LPS stimulated cyclooxygenase-2 expression in murine and human macrophages. These findings implicate ILK as a potential target for anti-inflammatory applications.

L8 ANSWER 2 OF 8 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2001:504909 BIOSIS
DOCUMENT NUMBER: PREV200100504909
TITLE: The **integrin linked kinase** (ILK) regulates inducible nitric oxide synthase expression and nitric oxide production in an NFkappaB-dependent manner.
AUTHOR(S): Tan, C. (1); Miu, A. (1); Dedhar, S. (1)
CORPORATE SOURCE: (1) Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, BC, V6H 3Z6 Canada
SOURCE: Inflammation Research, (September, 2001) Vol. 50 , No. Supplement 3, pp. S163. print.
Meeting Info.: 5th World Congress on Inflammation Edinburgh, Scotland September 22-26, 2001
ISSN: 1023-3830.
DOCUMENT TYPE: Conference
LANGUAGE: English
SUMMARY LANGUAGE: English

L8 ANSWER 3 OF 8 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2001:511057 BIOSIS
DOCUMENT NUMBER: PREV200100511057
TITLE: Anti-inflammatory properties of **integrin-linked kinase** 1 (ILK1) inhibitors.
AUTHOR(S): Kojic, L. (1); Zhang, J. (1); Goutsos, T. (1); Sanghera, A.

L12 ANSWER 1 OF 12 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1

ACCESSION NUMBER: 2002:377203 BIOSIS
DOCUMENT NUMBER: PREV200200377203
TITLE: Monocyte chemoattractant protein-1-induced activation of p42/44 MAPK and c-Jun in murine peritoneal macrophages: A potential pathway for macrophage activation.
AUTHOR(S): Sodhi, Ajit (1); Biswas, Subhra K.
CORPORATE SOURCE: (1) School of Biotechnology, Faculty of Science, Banaras Hindu University, Varanasi, 221005: ajit.sodhi@lycos.com India
SOURCE: Journal of Interferon and Cytokine Research, (May, 2002) Vol. 22, No. 5, pp. 517-526. http://www.liebertpub.com/JIR. print.
ISSN: 1079-9907.
DOCUMENT TYPE: Article
LANGUAGE: English

AB The role of monocyte chemoattractant protein-1 (MCP-1) in mediating the infiltration and activation of monocytes/macrophages into the sites of **inflammation** or tumor growth is well documented, but the molecular mechanism(s) involved in the process is poorly understood. In the current investigation, we demonstrate activation of the p42/44 MAPK-mediated signal transduction in murine peritoneal macrophages on stimulation with MCP-1 (10-100 ng/ml) in vitro. The p42/44 MAPK activation was determined by studying the expression of the phosphorylated p42/44 MAPK (Thr202/Tyr204) in the MCP-1-treated macrophages. This response was found to be rapid and time dependent, detectable within 5 min of MCP-1 stimulation. PD98058 (5-50 muM), a specific inhibitor of MAPK kinase (MEK) inhibited the p42/44 MAPK phosphorylation, indicating the specificity of the response. Furthermore, the MCP-1-induced phosphorylation of p42/44 MAPK was found to be blocked by pertussis toxin (100 ng/ml), tyrosine kinase inhibitor-genestein (10 ng/ml), **PI3K** inhibitor-**wortmannin** (20-200 muM), and anti-CCR2 antibody (2.5 mug/ml). Additionally, phosphorylation of JNK and activation of the transcription factor, c-Jun, were also noted in response to MCP-1 treatment. Lastly, the MCP-1-induced p42/44 MAPK activity was correlated with the functional activation of macrophages by demonstrating the dose-specific inhibition of actin polymerization, macrophage-mediated tumor cell cytotoxicity, and tumor necrosis factor-alpha (TNF-alpha) transcription/production afforded by PD98059 in the MCP-1-treated macrophages. Taken together, these data suggest the involvement of the p42/44 MAPK/c-Jun pathway in the signal transduction process, leading to activation of murine peritoneal macrophages.

L12 ANSWER 2 OF 12 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 2

ACCESSION NUMBER: 2002:185762 BIOSIS
DOCUMENT NUMBER: PREV200200185762
TITLE: fMLP-induced in vitro nitric oxide production and its regulation in murine peritoneal macrophages.
AUTHOR(S): Sodhi, Ajit (1); Biswas, Subhra K.
CORPORATE SOURCE: (1) Ajit Sodhi, School of Biotechnology, Banaras Hindu University, Varanas, 221005: ajit.sodhi@lycos.com India
SOURCE: Journal of Leukocyte Biology, (February, 2002) Vol. 71, No. 2, pp. 262-270. http://www.jleukbio.org. print.
ISSN: 0741-5400.
DOCUMENT TYPE: Article
LANGUAGE: English

AB Bacterial N-formyl peptides such as N-formyl-methionyl-leucyl-phenylalanine (fMLP) are important mediators of monocyte/macrophage recruitment and activation at the sites of **inflammation**. In the current study, the role of nitric oxide (NO) in the activation of murine peritoneal macrophages to tumoricidal state in response to in vitro fMLP treatment has been investigated. Murine peritoneal macrophages on

treatment with fMLP showed a dose- and time-dependent production of NO together with increased tumoricidal activity against P815 mastocytoma cells. L-NMMA, a specific inhibitor of L-arginine pathway, inhibited the fMLP-induced NO secretion and macrophage-mediated tumoricidal activity against P815 cells. These results indicate the L-arginine-dependent production of NO to be one of the effector mechanisms contributing to the tumoricidal activity of fMLP-treated macrophages. The expression of iNOS protein and iNOS mRNA is also observed. The pharmacological inhibitors genistein, **wortmannin**, H7, PD98059, TPCK, and pertussis toxin (PTX) blocked the fMLP-induced NO production, suggesting the involvement of tyrosine kinases, **PI3K**, PKC, p42/44 MAPkinase, NF-kappaB, and G-proteins. The expression of phospho-p42/44 MAPK and phospho-IkappaB was also observed. The role of protein phosphatases in the above pathway has been suggested using the specific inhibitors of these phosphatases, i.e., okadaic acid and sodium orthovanadate.

L12 ANSWER 3 OF 12 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 2000417593 MEDLINE
DOCUMENT NUMBER: 20393882 PubMed ID: 10933809
TITLE: Recruitment and activation of Raf-1 kinase by nitric oxide-activated Ras.
AUTHOR: Deora A A; Hajjar D P; Lander H M
CORPORATE SOURCE: Departments of Biochemistry, Weill Medical College of Cornell University, New York, New York 10021, USA.
CONTRACT NUMBER: AI37637 (NIAID)
GM55509 (NIGMS)
HL49666 (NHLBI)
+
SOURCE: BIOCHEMISTRY, (2000 Aug 15) 39 (32) 9901-8.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200009
ENTRY DATE: Entered STN: 20000915
Last Updated on STN: 20021219
Entered Medline: 20000905

AB Nitric oxide (NO) and related species serve as cellular messengers in various physiological and pathological processes. The monomeric G protein, Ras, transduces multiple signaling pathways with varying biological responses. We have previously reported that NO triggers Ras activation and recruitment of an effector, phosphatidylinositol 3'-kinase (**PI3K**) and Ras-dependent activation of mitogen-activated protein (MAP) kinases which include extracellular signal regulated kinases (ERKs), c-Jun NH(2)-terminal kinase (JNK), and p38 MAP kinase. In this study, we further defined NO-activated Ras signaling pathways. We have identified Raf-1 as another effector recruited by NO-activated Ras in T lymphocytes. NO activation results in association of Ras and Raf-1 and is biologically significant, as we observe an NO-induced increase in Raf-1 kinase activity. Downstream to Raf-1 kinase lie MAP kinases and their subsequent downstream targets, transcription factors. We found that treatment of T lymphocytes with NO yielded phosphorylation of the transcription factor, Elk-1. This phoshorylation is dependent on NO binding to the cysteine 118 residue of Ras. By further delineating the pathway with pharmacological inhibitors, Elk-1 phosphorylation was also found to be dependent on **PI3K** and ERK. Moreover, NO triggered an increase in mRNA levels of the proinflammatory cytokine, tumor necrosis factor-alpha (TNF-alpha), which was ERK dependent. Thus, we have defined an NO-induced signaling pathway in T lymphocytes arising at the membrane where NO-activated Ras recruits Raf-1 and culminating in the nucleus where Elk-1 is phosphorylated and TNF-alpha messenger RNA is induced. This NO-activated Ras-mediated signaling pathway may play a critical role in Elk-1-induced transcriptional activation of T lymphocytes, host defense and

inflammation.

L12 ANSWER 4 OF 12 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 2001145587 MEDLINE
DOCUMENT NUMBER: 20553205 PubMed ID: 11099401
TITLE: Mitogenic action of lysophosphatidic acid in proximal tubular epithelial cells obtained from voided human urine.
AUTHOR: Kumagai N; Inoue C N; Kondo Y; Iinuma K
CORPORATE SOURCE: Department of Pediatrics, Tohoku University School of Medicine, 1-1 Seiryo-machi, Aoba-ku, Sendai 980-8574, Japan.
SOURCE: CLINICAL SCIENCE, (2000 Dec) 99 (6) 561-7.
Journal code: 7905731. ISSN: 0143-5221.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200103
ENTRY DATE: Entered STN: 20010404
Last Updated on STN: 20021218
Entered Medline: 20010315

AB Focal tubular cell multiplication at sites on an injured nephron is a critical event in the recovery phase following acute tubular necrosis. During this process, numerous viable tubular cells exfoliate and are shed into the urine. Lysophosphatidic acid (LPA) is generated in the plasma membrane of injured cells and acts as an intercellular mediator of various biological processes, including **inflammation**, proliferation and repair. In the present study, exfoliated proximal tubule (PT) cells were isolated from human urine and the mitogenic effects of LPA were investigated as a model of repair and proliferation following renal injury. LPA stimulated a 23. 5% increase in DNA synthesis, a 29.4% increase in cell number and an 86.6% decrease in cAMP content. All of these responses were pertussis toxin sensitive, indicating the involvement of G(i)-type G-proteins in LPA signalling. Conversely, the LPA-induced DNA synthesis and the decrease in intracellular cAMP content were insensitive to **wortmannin**, an inhibitor of phosphatidylinositol 3-kinase (**PI3K**), suggesting a mitogenic response via **PI3K** -independent mechanisms. Furthermore, we detected specific mRNA transcripts for the recently cloned human LPA-receptors, endothelial differentiation gene (Edg)-2 and Edg-4 (Edg-2>>Edg-4) by reverse transcription-PCR in PT cells. Our data suggest that LPA may behave as a local growth factor in PT cells following tubular injury.

L12 ANSWER 5 OF 12 MEDLINE
ACCESSION NUMBER: 2002319445 MEDLINE
DOCUMENT NUMBER: 22055361 PubMed ID: 12060491
TITLE: In vitro activation of murine peritoneal macrophages by monocyte chemoattractant protein-1: upregulation of CD11b, production of proinflammatory cytokines, and the signal transduction pathway.
AUTHOR: Biswas Subhra K; Sodhi Ajit
CORPORATE SOURCE: School of Biotechnology, Banaras Hindu University, Varanasi 221005, India.
SOURCE: JOURNAL OF INTERFERON AND CYTOKINE RESEARCH, (2002 May) 22 (5) 527-38.
Journal code: 9507088. ISSN: 1079-9907.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200212
ENTRY DATE: Entered STN: 20020614
Last Updated on STN: 20021217
Entered Medline: 20021204

AB The CC chemokine monocyte chemotactic protein-1 (MCP-1) is a major mediator of monocyte/macrophage infiltration at the inflammatory sides under both physiologic and pathologic conditions. We report the ability of MCP-1 to activate murine peritoneal macrophages in vitro for enhanced expression of CD11b, macrophage-mediated cytotoxicity, and production of tumor necrosis factor-alpha (TNF-alpha) and interleukin-1 (IL-1). The macrophages treated with MCP-1 in vitro displayed significant cytolytic activity toward TNF-alpha-sensitive L929 cells in a dose-dependent manner. The macrophage-mediated L929 cytotoxicity was blocked in the presence of anti-TNF-alpha antibodies, suggesting the involvement of TNF-alpha. Production of TNF-alpha and IL-1 macrophages on MCP-1 treatment was maximum at 24 h of incubation with 100 ng/ml MCP-1. Enhanced TNF-alpha and IL-1beta mRNA expression was also demonstrated by RT-PCR, which revealed transcription of interferon gamma (IFN-gamma), IL-12, and related T cell-specific chemokine genes, KC and IP-10, in the MCP-1-treated macrophages. The pharmacologic inhibitors pertussis toxin (100 ng/ml), **wortmannin** (200 ng/ml), H-7 (10 microM), PD98059 (25 microM), and genistein (10 microg/ml) significantly inhibited TNF-alpha and IL-1 production in the MCP1-treated macrophages, suggesting the involvement of G-proteins, phosphoinositol-3-kinase (**PI3K**), protein kinase C, p42/44 MAPK, and tyrosine kinases in this process.

L12 ANSWER 6 OF 12 MEDLINE
ACCESSION NUMBER: 2001341643 MEDLINE
DOCUMENT NUMBER: 21240432 PubMed ID: 11342456
TITLE: Phosphoinositide 3-kinase modulation of beta(3)-integrin represents an endogenous "braking" mechanism during neutrophil transmatrix migration.
AUTHOR: Bruyninckx W J; Comerford K M; Lawrence D W; Colgan S P
CORPORATE SOURCE: Center for Experimental Therapeutics and Reperfusion Injury, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, USA.
CONTRACT NUMBER: DK50189 (NIDDK)
HL60569 (NHLBI)
PO-1 DE13499 (NIDCR)
SOURCE: BLOOD, (2001 May 15) 97 (10) 3251-8.
Journal code: 7603509. ISSN: 0006-4971.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 200106
ENTRY DATE: Entered STN: 20010618
Last Updated on STN: 20010618
Entered Medline: 20010614

AB During episodes of **inflammation**, neutrophils (polymorphonuclear leukocytes [PMNs]) encounter subendothelial matrix substrates that may require additional signaling pathways as directives for movement through the extracellular space. Using an in vitro endothelial and epithelial model, inhibitors of phosphoinositide 3-kinase (**PI3K**) were observed to promote chemoattractant-stimulated migration by as much as 8 +/- 0.3-fold. Subsequent studies indicated that PMNs respond in a similar manner to RGD-containing matrix substrates and that PMN-matrix interactions are potently inhibited by antibodies directed against beta(3)- but not beta(1)-integrin antibodies, and that **PI3K** inhibitors block beta(3)-integrin dependence. Biochemical analysis of intracellular beta(3)-integrin uncoupling by **PI3K** inhibitors revealed diminished beta(3)-integrin tyrosine phosphorylation and decreased association with p72(syk). Similarly, the p72(syk) inhibitor piceatannol promoted PMN transmatrix migration, whereas HIV-tat peptide-facilitated loading of peptides corresponding to the beta(3)-integrin cytoplasmic tail identified the functional tyrosine residues for this activity. These data indicate that **PI3K** -regulated beta(3)-integrin represents a natural "braking" mechanism for

PMNs during transit through the extracellular matrix.

L12 ANSWER 7 OF 12 MEDLINE
ACCESSION NUMBER: 2000496125 MEDLINE
DOCUMENT NUMBER: 20435835 PubMed ID: 10844001
TITLE: Factor VIIa/tissue factor-induced signaling via activation of Src-like kinases, phosphatidylinositol 3-kinase, and Rac.
AUTHOR: Versteeg H H; Hoedemaeker I; Diks S H; Stam J C; Spaargaren M; van Bergen En Henegouwen P M; van Deventer S J; Peppelenbosch M P
CORPORATE SOURCE: Laboratory for Experimental Internal Medicine, G2-130, Academic Medical Center, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands,.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (2000 Sep 15) 275 (37) 28750-6.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200010
ENTRY DATE: Entered STN: 20001027
Last Updated on STN: 20001027
Entered Medline: 20001013

AB Tissue factor (TF), apart from activating the extrinsic pathway of the blood coagulation, is a principal regulator of embryonic angiogenesis and oncogenic neoangiogenesis, but also influences **inflammation**, leukocyte diapedesis and tumor progression. The intracellular domain of TF lacks homology to other classes of receptors and hence the signaling mechanism is poorly understood. Here we demonstrate that factor VIIa (the natural ligand for TF) induces the activation of the Src family members c-Src, Lyn, and Yes, and subsequently phosphatidylinositol 3-kinase (**PI3K**), followed by stimulation of c-Akt/protein kinase B as well as the small GTPases Rac and Cdc42. In turn Rac mediates p38 mitogen-activated protein (MAP) kinase activation and cytoskeletal reorganization, whereas factor VIIa-induced p42/p44 MAP kinase stimulation required **PI3K** enzymatic activity but was not inhibited by dominant negative Rac proteins. We propose that this Src family member/ **PI3K**/Rac-dependent signaling pathway is a major mediator of factor VIIa/TF effects in pathophysiology.

L12 ANSWER 8 OF 12 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 3
ACCESSION NUMBER: 2001:251417 CAPLUS
DOCUMENT NUMBER: 135:30354
TITLE: P2Y2 nucleotide receptor signaling in human monocytic cells: activation, desensitization, and coupling to mitogen-activated protein kinases
AUTHOR(S): Santiago-Perez, Laura I.; Flores, Rosa V.; Santos-Berrios, Cynthia; Chorna, Nataliya E.; Krugh, Brent; Garrad, Richard C.; Erb, Laurie; Weisman, Gary A.; Gonzalez, Fernando A.
CORPORATE SOURCE: Department of Chemistry, University of Puerto Rico, San Juan, 00931-3346, P. R.
SOURCE: Journal of Cellular Physiology (2001), 187(2), 196-208
CODEN: JCLLAX; ISSN: 0021-9541
PUBLISHER: Wiley-Liss, Inc.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Activation of P2Y2 receptors by extracellular nucleotides has been shown to induce phenotypic differentiation of human pro-monocytic U937 cells that is assocd. with the inflammatory response. The P2Y2 receptor agonist, UTP, induced the phosphorylation of the MAP kinases MEK1/2 and ERK1/2 in a sequential manner, since ERK1/2 phosphorylation was abolished

by the MEK1/2 inhibitor PD 098059. Other results indicated that P2Y2 receptors can couple to MAP kinases via phosphatidylinositol 3-kinase (**PI3K**) and c-src. Accordingly, ERK1/2 phosphorylation induced by UTP was inhibited by the **PI3K** inhibitors, **wortmannin** and LY294002, and the c-src inhibitors, radicicol and PP2, but not by inhibitors of protein kinase C (PKC). The phosphorylation of ERK1/2 was independent of the ability of P2Y2 receptors to increase the concn. of intracellular free calcium, since chelation of intracellular calcium by BAPTA did not diminish the phosphorylation of ERK1/2 induced by UTP. A 5-min treatment with UTP reduced U937 cell responsiveness to a subsequent UTP challenge. UTP-induced desensitization was characterized by an increase in the EC50 for receptor activation (from 0.44 to 9.3 .mu.M) and a dramatic (.apprx.75%) decrease in the maximal calcium mobilization induced by a supramaximal dose of UTP. Phorbol ester treatment also caused P2Y2 receptor desensitization (EC50 = 12.3 .mu.M UTP and maximal calcium mobilization reduced by .apprx.33%). The protein kinase C inhibitor GF 109203X failed to significantly inhibit the UTP-induced desensitization of the P2Y2 receptor, whereas the protein phosphatase inhibitor okadaic acid blocked receptor resensitization. Recovery of receptor activity after UTP-induced desensitization was evident in cells treated with agonist for 5 or 30 min. However, P2Y2 receptor activity remained partially desensitized 30 min after pretreatment of cells with UTP for 1 h or longer. This sustained desensitized state correlated with a decrease in P2Y2 receptor mRNA levels. Desensitization of ERK1/2 phosphorylation was induced by a 5-min pretreatment with UTP, and cell responsiveness did not return even after a 30-min incubation of cells in the absence of an agonist. Results suggest that desensitization of the P2Y2 receptor may involve covalent modifications (i.e., receptor phosphorylation) that functionally uncouple the receptor from the calcium signaling pathway, and that transcriptional regulation may play a role in long-term desensitization. Our results indicate that calcium mobilization and ERK1/2 phosphorylation induced by P2Y2 receptor activation are independent events in U937 monocytes.

REFERENCE COUNT: 54 THERE ARE 54 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 9 OF 12 CAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 2001:804661 CAPLUS

DOCUMENT NUMBER: 137:62068

TITLE: Signaling pathway of activation of NDAPH oxidase in HL-60 cell with fMLP stimulation

AUTHOR(S): Cui, Yudong; Osamu, Inanami; Tohru, Yamamori; Mikinori, Kuwabara

CORPORATE SOURCE: Center of Gene Engineering, Heilongjiang August First Agricultural University, Mishan, 158308, Peop. Rep. China

SOURCE: Mianyixue Zazhi (2001), 17(5), 359-363
CODEN: MIZAED; ISSN: 1000-8861

PUBLISHER: Mianyixue Zazhi Bianjibu

DOCUMENT TYPE: Journal

LANGUAGE: Chinese

AB The effects of intracellular Ca2+([Ca2+]i) and kinases on the activation of NADPH oxidase and the signaling pathways of activation of NADPH oxidase in neutrophils were studied. The influence of [Ca2+]i and some kinases on the activation of NADPH oxidase were studied in differentiated HL-60 with fMLP stimulation. 10 .mu.Mol/L BAPTA-AM significantly attenuated the chemotactic peptide formyl-methionyl-leucyl-phenylalanine (fMLP)- induced O-2 generation. 8.mu.Mol/L GF109293x remarkably inhibited O-2 generation. 50.mu.Mol/L SB203580, 50.mu.mol/L PD98059 and 0.1.mu.mol/L **wortmannin** suppressed O-2 generation in degree. The activation of PKC, **PI3K**, p38 and ERK was not essential to [Ca2+]i elevation. Certain effects of [Ca2+]i, PKC and **PI3K** on the activation of p38 were obsd., but the activation of ERK and Akt were mainly regulated by PI3-K. These results suggested that both [Ca2+]i-dependent pathway (PKC)

and [Ca2+]i-independent pathway (PI3-K, p38, and ERK) were both important in the activation of NADPH oxidase.

L12 ANSWER 10 OF 12 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2001:669396 CAPLUS
DOCUMENT NUMBER: 136:293430
TITLE: Signaling mechanism for equine neutrophil activation by immune complexes
AUTHOR(S): Jones, S. L.; Sharief, Y.; Chilcoat, C. D.
CORPORATE SOURCE: College of Veterinary Medicine, Department of Clinical Sciences, North Carolina State University, Raleigh, NC, 27606, USA
SOURCE: Veterinary Immunology and Immunopathology (2001), 82(1-2), 87-100
CODEN: VIIMDS; ISSN: 0165-2427
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Neutrophils (PMN) are crit. host defense cells that have a role in the pathophysiol. of a variety of inflammatory diseases, particularly those diseases assocd. with antigen-antibody immune complexes (IC) deposited in tissues. Activation of PMN by IC is most efficient if the IC are presented immobilized on a surface. Adhesion to the immobilized IC is important for subsequent activation of PMN effector functions, such as generation of reactive oxygen metabolites. Adhesion of human PMN to immobilized IC requires the expression and activation of adhesion receptors called integrins. Of the integrins expressed on PMN, the .beta.2 family has been of particular importance for PMN function. The mechanism of .beta.2 integrin activation during adhesion to IC has been studied in human PMN, but not in equine PMN. The authors show here that adhesion of equine PMN to immobilized IC requires .beta.2 integrins. Like adhesion, IC-induced respiratory burst activity is dependent on .beta.2 integrins. Furthermore, the signaling pathway triggering .beta.2 integrin-dependent adhesion of equine PMN to IC and subsequent generation of respiratory burst activity is inhibited by the specific phosphatidylinositol 3-kinase (**PI3K**) antagonists **wortmannin** and LY294002 with IC50 (concn. at which 50% inhibition is achieved) similar to the published values for inhibition of **PI3K** enzymic activity. In contrast, PMA-induced activation of .beta.2 integrin-dependent adhesion and respiratory burst activity are **wortmannin** and LY294002 insensitive. Thus, like in human PMN, IC-induced activation of .beta.2 integrins and .beta.2 integrin-dependent functions in equine PMN are dependent on **PI3K** activity.
REFERENCE COUNT: 33 THERE ARE 33 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 11 OF 12 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 2003086362 EMBASE
TITLE: IKK.alpha. regulates mitogenic signaling through transcriptional induction of cyclin D1 via Tcf.
AUTHOR: Albanese C.; Wu K.; D'Amico M.; Jarrett C.; Joyce D.; Hughes J.; Hulit J.; Sakamaki T.; Fu M.; Ben-Ze'ev A.; Bromberg J.F.; Lamberti C.; Verma U.; Gaynor R.B.; Byers S.W.; Pestell R.G.
CORPORATE SOURCE: R.G. Pestell, Department of Oncology, Lombardi Cancer Center, Georgetown Univ. School of Medicine, Washington, DC 20007, United States. pestell@georgetown.edu
SOURCE: Molecular Biology of the Cell, (1 Feb 2003) 14/2 (585-599). Refs: 79
ISSN: 1059-1524 CODEN: MBCEEV
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 016 Cancer
029 Clinical Biochemistry

037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The Wnt/.beta.-catenin/Tcf and I.kappa.B/NF-.kappa.B cascades are independent pathways involved in cell cycle control, cellular differentiation, and **inflammation**. Constitutive Wnt/.beta.-catenin signaling occurs in certain cancers from mutation of components of the pathway and from activating growth factor receptors, including RON and MET. The resulting accumulation of cytoplasmic and nuclear .beta.-catenin interacts with the Tcf/LEF transcription factors to induce target genes. The I.kappa.B kinase complex (IKK) that phosphorylates I.kappa.B contains IKK.alpha., IKK.beta., and IKK.gamma.. Here we show that the cyclin D1 gene functions as a point of convergence between the Wnt/.beta.-catenin and I.kappa.B pathways in mitogenic signaling. Mitogenic induction of G(1)-S phase progression and cyclin D1 expression was **PI3K** dependent, and cyclin D1(-/-) cells showed reduced **PI3K**-dependent S-phase entry. **PI3K**-dependent induction of cyclin D1 was blocked by inhibitors of **PI3K** /Akt/I.kappa.B/IKK.alpha. or .beta.-catenin signaling. A single Tcf site in the cyclin D1 promoter was required for induction by **PI3K** or IKK.alpha.. In IKK.alpha.(-/-) cells, mitogen-induced DNA synthesis, and expression of Tcf-responsive genes was reduced. Reintroduction of IKK.alpha. restored normal mitogen induction of cyclin D1 through a Tcf site. In IKK.alpha.(-/-) cells, .beta.-catenin phosphorylation was decreased and purified IKK.alpha. was sufficient for phosphorylation of .beta.-catenin through its N-terminus in vitro. Because IKK.alpha. but not IKK.alpha. induced cyclin D1 expression through Tcf activity, these studies indicate that the relative levels of IKK.alpha. and IKK.beta. may alter their substrate and signaling specificities to regulate mitogen-induced DNA synthesis through distinct mechanisms.

L12 ANSWER 12 OF 12 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 2003088530 EMBASE
TITLE: Phosphoinositide 3-kinase .gamma.: A key modulator in **inflammation** and allergy.
AUTHOR: Wymann M.P.; Bjorklof K.; Calvez R.; Finan P.; Thomas M.; Trifilieff A.; Barbier M.; Altruda F.; Hirsch E.; Laffargue M.
CORPORATE SOURCE: M.P. Wymann, Department of Medicine, University of Fribourg, Rue du Musee 5, CH-1700 Fribourg, Switzerland. Matthias.Wymann@UniFR.ch
SOURCE: Biochemical Society Transactions, (2003) 31/1 (275-280). Refs: 54
ISSN: 0300-5127 CODEN: BCSTB5
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Conference Article
FILE SEGMENT: 005 General Pathology and Pathological Anatomy
026 Immunology, Serology and Transplantation
029 Clinical Biochemistry
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Chronic **inflammation** and allergy involve the activation of tissue-resident cells and, later on, the invasion of effector cells. We have previously shown that the loss of phosphoinositide 3-kinase (**PI3K**) .gamma. impairs chemokine-dependent migration of neutrophils and macrophages both in vitro and in vivo. On the other hand, **PI3K** .gamma. is not required either during phagocytic processes or in the activation of bactericidal activities like granule secretion and particle-mediated respiratory burst in neutrophils. Tissue mast cells are key regulators in allergy and **inflammation** and release histamine upon clustering of their IgE receptors. We have demonstrated that murine mast cell responses are exacerbated in vitro and in vivo by autocrine signals, and require functional **PI3K**.gamma.. Adenosine, acting

through the A(3) adenosine receptor, as well as other agonists of G(.alpha.i)-coupled receptors, transiently increased Ptdlns(3,4,5)P(3) exclusively via **PI3K**.gamma.. **PI3K**.gamma.-derived Ptdlns(3,4,5)P(3) was instrumental for initiation of a sustained influx of external Ca(2+) and degranulation. Mice that lacked **PI3K**.gamma. did not form oedema when challenged by passive systemic anaphylaxis. **PI3K**.gamma. thus relays inflammatory signals through various GPCRs, and is thus central to mast cell function. Taken together, this suggests that pharmaceutical targeting of **PI3K**.gamma. might alleviate **inflammation** at both early and late stages of the allergic response.

L11 ANSWER 1 OF 8 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1
ACCESSION NUMBER: 2003:104482 BIOSIS
DOCUMENT NUMBER: PREV200300104482
TITLE: Negative regulation of phosphatidylinositol 3-kinase and Akt signalling pathway by PKC.
AUTHOR(S): Wen, Hui C.; Huang, Wan C.; Ali, Adnan; Woodgett, James R.; Lin, Wan W. (1)
CORPORATE SOURCE: (1) Department of Pharmacology, College of Medicine, National Taiwan University, Taipei, Taiwan: wwl@ha.mc.ntu.edu.tw Taiwan
SOURCE: Cellular Signalling, (January 2003, 2003) Vol. 15, No. 1, pp. 37-45. print.
ISSN: 0898-6568.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Although substantial studies have begun to explore the regulation of phosphatidylinositol 3-kinase/Akt cascade by different signalling pathways, whether protein kinase C (PKC) activity plays a crucial role remains as yet unclear. In this study, we found that in A549 and HEK293 cells non-selective PKC inhibitors Ro 31-8220 and bisindolylmaleimide VIII, and PKCbeta inhibitor LY 379196, caused Akt/PKB phosphorylation at Ser 473 and increased the upstream activator, **integrin-linked kinase** (ILK) activity. The increased Akt phosphorylation was blocked by phosphatidylinositol 3-kinase inhibitor **wortmannin** and the newly identified PIP3-dependent kinases (PDK) inhibitor SB 203580. In contrast to the Akt stimulation caused by PKC inhibitors, PMA attenuated Akt/PKB phosphorylation. We also found that this stimulating effect on Akt phosphorylation by PKC inhibitors was not the result of phosphatase inhibition, since treatment with PP2A, PP2B and tyrosine phosphatase inhibitors (okadaic acid, FK506 and sodium orthovanadate, respectively) had no effect. We conclude that phosphatidylinositol 3-kinase/Akt signalling pathway is regulated by PKC in a negative manner.

L11 ANSWER 2 OF 8 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 2
ACCESSION NUMBER: 2002:451376 BIOSIS
DOCUMENT NUMBER: PREV200200451376
TITLE: Evidence that the platelet integrin alphaIIbbeta3 is regulated by the **Integrin-Linked Kinase**, ILK, in a PI3-kinase dependent pathway.
AUTHOR(S): Pasquet, Jean-Max (1); Noury, Malia; Nurden, Alan T.
CORPORATE SOURCE: (1) UMR 5533 CNRS, Hopital Cardiologique du Haut-Leveque, Avenue Magellan, 33604, Pessac: jean-max.pasquet@umr5533.u-bordeaux2.fr France
SOURCE: Thrombosis and Haemostasis, (July, 2002) Vol. 88, No. 1, pp. 115-122. http://www.thrombosis-online.com. print.
ISSN: 0340-6245.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Platelet aggregation is mediated by the integrin alphaIIbbeta3 which is activated by intracellular signals during platelet activation. We have attempted to determine if ILK ("**Integrin-Linked Kinase**") is involved in the regulation of alphaIIbbeta3 function. ILK co-immunoprecipitated with beta3 in stimulated platelets. Using confocal microscopy, ILK was detected in the cytoplasm of resting platelets. ADP or PMA stimulation led to its translocation to the plasma membrane. In parallel, there was a transient increase in ILK kinase activity, association with and phosphorylation of beta3. Inhibition of PI3-kinase by two unrelated inhibitors (**wortmannin** and LY294002) prevented ILK-related functions. However, it did not prevent the conformational change in alphaIIbbeta3 (shown by PAC-1 binding), although integrin affinity for fibrinogen was decreased as measured using FITC-fibrinogen. Furthermore, aggregate formation was reduced. Thus ILK transiently associates with and phosphorylates beta3 in a PI3-kinase

dependent manner suggesting that it participates at an intermediate stage in a critical mechanism for assuring large stable aggregates.

L11 ANSWER 3 OF 8 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 3
ACCESSION NUMBER: 2000:135694 BIOSIS
DOCUMENT NUMBER: PREV200000135694
TITLE: **Integrin-linked kinase** regulates phosphorylation of serine 473 of protein kinase B by an indirect mechanism.
AUTHOR(S): Lynch, Danielle K.; Ellis, Christine A.; Edwards, Paul A. W.; Hiles, Ian D. (1)
CORPORATE SOURCE: (1) Molecular Pharmacology Unit, Medicines Research Centre, GlaxoWellcome Research and Development, Gunnels Wood Road, Stevenage, SG1 2NY UK
SOURCE: Oncogene., (Dec. 23, 1999) Vol. 18, No. 56, pp. 8024-8032. ISSN: 0950-9232.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The serine threonine kinase protein kinase B regulates cellular activities as diverse as glycogen metabolism and apoptosis. Full activation of protein kinase B requires 3-phosphoinositides and dual phosphorylation on threonine-308 and serine-473. CaM-K kinase and 3-phosphoinositide dependent-kinase-1 phosphorylate threonine-308. **Integrin-linked kinase** reportedly phophorylates serine-473. Consistent with this, in a model COS cell system we show that expression of wild-type **integrin-linked kinase** promotes the **wortmannin** sensitive phosphorylation of serine-473 of protein kinase B and its downstream substrates, and inhibits C2-ceramide induced apoptosis. In contrast, **integrin-linked kinase** mutated in a lysine residue critical for function in protein kinases is inactive in these experiments, and furthermore, acts dominantly to block serine-473 phosphorylation induced by ErbB4. However, alignment of analogous sequences from different species demonstrates that **integrin-linked kinase** is not a typical protein kinase and identifies a conserved serine residue which potentially regulates kinase activity in a phosphorylation dependent manner. Mutation of this serine to aspartate or glutamate, but not alanine, in combination with the inactivating lysine mutation restores **integrin-linked kinase** dependent phosphorylation of serine-473 of protein kinase B. These data strongly suggest that **integrin-linked kinase** does not possess serine-473 kinase activity but functions as an adaptor to recruit a serine-473 kinase or phosphatase.

L11 ANSWER 4 OF 8 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:241414 BIOSIS
DOCUMENT NUMBER: PREV200200241414
TITLE: Regulation of the affinity state of the integrin alphaIIbbeta3 in platelets by the **integrin-linked kinase**, ILK, in a PI3-kinase dependent pathway.
AUTHOR(S): Pasquet, Jean-Max G. (1); Noury, Malia (1); Nurden, Alan T. (1)
CORPORATE SOURCE: (1) UMR 5533 CNRS, Pessac France
SOURCE: Blood, (November 16, 2001) Vol. 98, No. 11 Part 1, pp. 514a. http://www.bloodjournal.org/. print.
Meeting Info.: 43rd Annual Meeting of the American Society of Hematology, Part 1 Orlando, Florida, USA December 07-11, 2001
ISSN: 0006-4971.
DOCUMENT TYPE: Conference
LANGUAGE: English
AB Platelet aggregation plays a key role in hemostasis through the formation

of the hemostatic plug. This critical function is dependent on the integrin alphaIIbbeta3 which is activated by intracellular signals, called inside-out signaling, allowing fibrinogen binding. The occupied receptor in turn signals back into the cell (outside-in signaling), a process that leads to cytoskeletal modifications, the formation of multi-protein complexes and integrin clustering. Many of these events remain poorly understood. A role for proteins that interact with integrin cytoplasmic domains has been proposed both in inside-out and in outside-in signaling. One such potential protein, **integrin-linked kinase** (ILK) is known to be expressed in platelets. In an attempt to determine if ILK is involved in the function of alphaIIbbeta3, we have studied the subcellular localization of ILK, its association with the beta3 subunit, and its kinase activity in platelets before and after stimulation. ILK co-immunoprecipitated with alphaIIbbeta3 in ADP-, collagen-, TRAP-, thrombin-, A23187- and PMA-stimulated platelets, although the strongest association was detected for ADP and PMA. Using confocal microscopy, ILK was detected in the cytoplasm of resting platelets. ADP or PMA stimulation led to the translocation of ILK to the plasma membrane where it co-localized with alphaIIbbeta3. The detection of the kinase activity in ILK immunoprecipitates from NP-40 lysates using an in vitro kinase assay demonstrated a transient increase in ILK activity (with autophosphorylation), association with and phosphorylation of the beta3 subunit. These events with phosphorylation and dephosphorylation were clearly seen during reversible platelet aggregation with ADP. ILK possesses a pleckstrin homology domain which is involved in the regulation of its kinase activity through a PI3-kinase dependent pathway. Inhibition of PI3-kinase by two unrelated inhibitors (**wortmannin** and LY294002) prevented relocation of ILK in PMA-stimulated platelets, abolished ILK activation and its association with beta3. However, PI3-kinase inhibition did not prevent the conformational change in a alphaIIbbeta3 (checked through PAC-1 binding in flow cytometry), although it decreased the affinity of the integrin for fibrinogen as measured using FITC-fibrinogen. Furthermore aggregate formation was reduced. In summary, these results show that ILK transiently associates with and phosphorylates the beta3 subunit in a PI3-kinase dependent manner suggesting that ILK participates at an intermediate stage in a critical mechanism for assuring a stable and strong interaction with fibrinogen allowing stable aggregate formation.

L11 ANSWER 5 OF 8 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2001:462366 BIOSIS
DOCUMENT NUMBER: PREV200100462366
TITLE: The roles of **integrin-linked kinase** in Her-2/neu signaling pathways.
AUTHOR(S): Makino, Keishi (1); Hung, Mien-Chie (1)
CORPORATE SOURCE: (1) M.D. Anderson Cancer Center, Houston, TX USA
SOURCE: Proceedings of the American Association for Cancer Research Annual Meeting, (March, 2001) Vol. 42, pp. 297. print. Meeting Info.: 92nd Annual Meeting of the American Association for Cancer Research New Orleans, LA, USA March 24-28, 2001
ISSN: 0197-016X.
DOCUMENT TYPE: Conference
LANGUAGE: English
SUMMARY LANGUAGE: English

L11 ANSWER 6 OF 8 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2001:311557 BIOSIS
DOCUMENT NUMBER: PREV200100311557
TITLE: The regulation of **integrin-linked kinase** in human platelets.
AUTHOR(S): Thomas, Joanne M. (1); Barry, Fiona A. (1); Cicmil, Milenko (1); Sage, Tanya (1); Gibbins, Jonathan M. (1)
CORPORATE SOURCE: (1) School of Animal and Microbial Sciences, University of

Reading, Reading, Berkshire UK
SOURCE: Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 245a. print.
Meeting Info.: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000 American Society of Hematology
. ISSN: 0006-4971.
DOCUMENT TYPE: Conference
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The serine/threonine **integrin-linked kinase** (ILK) has been implicated in the regulation of cell: cell and cell: extracellular matrix (ECM) adhesion through its ability to interact with the cytoplasmic domains of the beta1, beta2, and beta3 integrin subunits. We have found that this kinase is expressed in human platelets and also in megakaryocytic cell lines raising the possibility of participation in signaling via receptors containing the beta1 or beta3 integrin subunits. Integrins are capable of bi-directional signal transduction, transmitting both outside-in and inside-out signals. This is exemplified in platelets by the fibrinogen receptor (alphaIIbbeta3) and recent reports suggest that the collagen adhesion receptor (alpha2beta1) may also be regulated by inside-out signaling. As a result of inside-out signaling, the affinity of alpha2beta1 for collagen increases following platelet activation with a range of agonists such as ADP, CRP and thrombin, and this activation is sensitive to a number of inhibitors including **wortmannin**. Interestingly, ILK has been implicated in the regulation of inside-out signaling in epithelial cells in response to a fibronectin substrate. The aim of this study was to determine whether ILK participates in signaling stimulated by a number of platelet agonists. Using a radioactive in vitro kinase assay we demonstrate that collagen, convulxin (a glycoprotein VI selective agonist) and thrombin modulate ILK kinase activity. Following an initial transient but substantial increase in kinase activity within 60 seconds of exposure to agonist, ILK activity decreases below that of resting platelets. Whilst the functional significance of the effects of collagen, convulxin and thrombin on the activity of ILK in platelets has yet to be determined, the possibility that ILK participates in inside-out signaling through alpha2beta1 in response to collagen is under investigation. Indeed, a kinase present in beta1 immunoprecipitates shows a very similar pattern of activity in an in vitro kinase assay. Furthermore, we can demonstrate an association between ILK and beta1 by co-immunoprecipitation from platelet cell lysates. Using immunofluorescence confocal microscopy, we present data on the co-localization of ILK with alpha2beta1 in platelets and megakaryocytic cells to sites of focal adhesion upon exposure to the ECM protein collagen. We propose that the modulation of ILK activity may underlie the agonist-induced conversion of alpha2beta1 to a high affinity conformation upon platelet activation.

L11 ANSWER 7 OF 8 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 1999382019 MEDLINE
DOCUMENT NUMBER: 99382019 PubMed ID: 10454216
TITLE: Mechanism of protein kinase B activation by insulin/insulin-like growth factor-1 revealed by specific inhibitors of phosphoinositide 3-kinase--significance for diabetes and cancer.
AUTHOR: Galetic I; Andjelkovic M; Meier R; Brodbeck D; Park J; Hemmings B A
CORPORATE SOURCE: Friedrich Miescher Institute, Basel, Switzerland.
SOURCE: PHARMACOLOGY AND THERAPEUTICS, (1999 May-Jun) 82 (2-3) 409-25. Ref: 198
Journal code: 7905840. ISSN: 0163-7258.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)

(REVIEW, ACADEMIC)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199910
ENTRY DATE: Entered STN: 20000111
Last Updated on STN: 20010730
Entered Medline: 19991026

AB Protein kinase B (PKB) is a member of the second messenger subfamily of protein kinases. The three isoforms of PKB identified have an amino-terminal pleckstrin homology domain, a central kinase domain, and a carboxy-terminal regulatory domain. PKB is the major downstream target of receptor tyrosine kinases that signal via the phosphoinositide (PI) 3-kinase. The crucial role of lipid second messengers in PKB activation has been dissected through the use of the PI 3-kinase-specific inhibitors **wortmannin** and LY294002. Receptor-activated PI 3-kinase synthesises the lipid second messenger PI-3,4,5-trisphosphate, leading to the recruitment of PKB to the membrane. Membrane attachment of PKB is mediated by its pleckstrin homology domain binding to PI-3,4,5-trisphosphate or PI-3,4-bisphosphate with high affinity. Activation of PKB alpha and beta is then achieved at the plasma membrane by phosphorylation of Thr308/309 in the A-loop of the kinase domain and Ser473/474 in the carboxy-terminal regulatory region, respectively. The upstream kinase that phosphorylates PKB on Thr308, termed PI-dependent protein kinase-1, has been identified and extensively characterised. A candidate for the Ser473/474 kinase, termed the **integrin-linked kinase**, has been identified recently. Activated PKB is implicated in glucose metabolism, transcriptional control, and in the regulation of apoptosis in many different cell types. Stimulation of PKB activity protects cells from apoptosis by phosphorylation and inactivation of the pro-apoptotic protein BAD. These results could explain why PKB is overexpressed in some ovarian, breast, and pancreatic carcinomas.

L11 ANSWER 8 OF 8 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 1998409636 MEDLINE
DOCUMENT NUMBER: 98409636 PubMed ID: 9736715
TITLE: Phosphoinositide-3-OH kinase-dependent regulation of glycogen synthase kinase 3 and protein kinase B/AKT by the **integrin-linked kinase.**
AUTHOR: Delcommenne M; Tan C; Gray V; Rue L; Woodgett J; Dedhar S
CORPORATE SOURCE: British Columbia Cancer Agency, Jack Bell Research Centre, 2660 Oak Street, Vancouver, British Columbia V6H 3Z6 Canada.
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1998 Sep 15) 95 (19) 11211-6. Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199810
ENTRY DATE: Entered STN: 19990106
Last Updated on STN: 20021218
Entered Medline: 19981026

AB **Integrin-linked kinase** (ILK) is an ankyrin-repeat containing serine-threonine protein kinase capable of interacting with the cytoplasmic domains of integrin beta1, beta2, and beta3 subunits. Overexpression of ILK in epithelial cells disrupts cell-extracellular matrix as well as cell-cell interactions, suppresses suspension-induced apoptosis (also called Anoikis), and stimulates anchorage-independent cell cycle progression. In addition, ILK induces nuclear translocation of beta-catenin, where the latter associates with a T cell factor/lymphocyte enhancer-binding factor 1 (TCF/LEF-1) to form an activated transcription factor. We now demonstrate that ILK activity is rapidly, but transiently, stimulated upon attachment of cells to

fibronectin, as well as by insulin, in a phosphoinositide-3-OH kinase [Pi(3)K]-dependent manner. Furthermore, phosphatidylinositol(3,4,5)trispho sphate specifically stimulates the activity of ILK in vitro, and in addition, membrane targetted constitutively active Pi(3)K activates ILK in vivo. We also demonstrate here that ILK is an upstream effector of the Pi(3)K-dependent regulation of both protein kinase B (PKB/AKT) and glycogen synthase kinase 3 (GSK-3). Specifically, ILK can directly phosphorylate GSK-3 in vitro and when stably, or transiently, overexpressed in cells can inhibit GSK-3 activity, whereas the overexpression of kinase-deficient ILK enhances GSK-3 activity. In addition, kinase-active ILK can phosphorylate PKB/AKT on serine-473, whereas kinase-deficient ILK severely inhibits endogenous phosphorylation of PKB/AKT on serine-473, demonstrating that ILK is involved in agonist stimulated, Pi(3)K-dependent, PKB/AKT activation. ILK is thus a receptor-proximal effector for the Pi(3)K-dependent, extracellular matrix and growth factor mediated, activation of PKB/AKT, and inhibition of GSK-3.

L12 ANSWER 1 OF 5 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1
ACCESSION NUMBER: 2002:451376 BIOSIS
DOCUMENT NUMBER: PREV200200451376
TITLE: Evidence that the platelet integrin alphaIIbbeta3 is regulated by the **Integrin-Linked Kinase**, ILK, in a PI3-kinase dependent pathway.
AUTHOR(S): Pasquet, Jean-Max (1); Noury, Malia; Nurden, Alan T.
CORPORATE SOURCE: (1) UMR 5533 CNRS, Hopital Cardiologique du Haut-Leveque, Avenue Magellan, 33604, Pessac: jean-max.pasquet@umr5533.u-bordeaux2.fr France
SOURCE: Thrombosis and Haemostasis, (July, 2002) Vol. 88, No. 1, pp. 115-122. http://www.thrombosis-online.com. print. ISSN: 0340-6245.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Platelet aggregation is mediated by the integrin alphaIIbbeta3 which is activated by intracellular signals during platelet activation. We have attempted to determine if ILK ("**Integrin-Linked Kinase**") is involved in the regulation of alphaIIbbeta3 function. ILK co-immunoprecipitated with beta3 in stimulated platelets. Using confocal microscopy, ILK was detected in the cytoplasm of resting platelets. ADP or PMA stimulation led to its translocation to the plasma membrane. In parallel, there was a transient increase in ILK kinase activity, association with and phosphorylation of beta3. Inhibition of PI3-kinase by two unrelated inhibitors (wortmannin and **LY294002**) prevented ILK-related functions. However, it did not prevent the conformational change in alphaIIbbeta3 (shown by PAC-1 binding), although integrin affinity for fibrinogen was decreased as measured using FITC-fibrinogen. Furthermore, aggregate formation was reduced. Thus ILK transiently associates with and phosphorylates beta3 in a PI3-kinase dependent manner suggesting that it participates at an intermediate stage in a critical mechanism for assuring large stable aggregates.

L12 ANSWER 2 OF 5 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:329922 BIOSIS
DOCUMENT NUMBER: PREV200200329922
TITLE: **Integrin linked kinase** 1 (ILK1) stimulates collecting tubule morphogenesis in vitro.
AUTHOR(S): Mahendra, Ahalya S. (1); Yu, Yunkai; Klamut, Henry; Rosenblum, Norman D.; Hannigan, Greg E. (1)
CORPORATE SOURCE: (1) Program Cell Biology, Hosp Sick Children, Toronto, ON Canada
SOURCE: Journal of the American Society of Nephrology, (September, 2001) Vol. 12, No. Program and Abstract Issue, pp. 597A. http://www.jasn.org/. print.
Meeting Info.: ASN (American Society of Nephrology)/ISN (International Society of Nephrology) World Congress of Nephrology San Francisco, CA, USA October 10-17, 2001
ISSN: 1046-6673.
DOCUMENT TYPE: Conference
LANGUAGE: English

L12 ANSWER 3 OF 5 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:241414 BIOSIS
DOCUMENT NUMBER: PREV200200241414
TITLE: Regulation of the affinity state of the integrin alphaIIbbeta3 in platelets by the **integrin-linked kinase**, ILK, in a PI3-kinase dependent pathway.
AUTHOR(S): Pasquet, Jean-Max G. (1); Noury, Malia (1); Nurden, Alan T. (1)
CORPORATE SOURCE: (1) UMR 5533 CNRS, Pessac France
SOURCE: Blood, (November 16, 2001) Vol. 98, No. 11 Part 1, pp. 514a. http://www.bloodjournal.org/. print.

Meeting Info.: 43rd Annual Meeting of the American Society of Hematology, Part 1 Orlando, Florida, USA December 07-11, 2001
ISSN: 0006-4971.
DOCUMENT TYPE: Conference
LANGUAGE: English

AB Platelet aggregation plays a key role in hemostasis through the formation of the hemostatic plug. This critical function is dependent on the integrin alphaIIbbeta3 which is activated by intracellular signals, called inside-out signaling, allowing fibrinogen binding. The occupied receptor in turn signals back into the cell (outside-in signaling), a process that leads to cytoskeletal modifications, the formation of multi-protein complexes and integrin clustering. Many of these events remain poorly understood. A role for proteins that interact with integrin cytoplasmic domains has been proposed both in inside-out and in outside-in signaling. One such potential protein, **integrin-linked kinase** (ILK) is known to be expressed in platelets. In an attempt to determine if ILK is involved in the function of alphaIIbbeta3, we have studied the subcellular localization of ILK, its association with the beta3 subunit, and its kinase activity in platelets before and after stimulation. ILK co-immunoprecipitated with alphaIIbbeta3 in ADP-, collagen-, TRAP-, thrombin-, A23187- and PMA-stimulated platelets, although the strongest association was detected for ADP and PMA. Using confocal microscopy, ILK was detected in the cytoplasm of resting platelets. ADP or PMA stimulation led to the translocation of ILK to the plasma membrane where it co-localized with alphaIIbbeta3. The detection of the kinase activity in ILK immunoprecipitates from NP-40 lysates using an in vitro kinase assay demonstrated a transient increase in ILK activity (with autophosphorylation), association with and phosphorylation of the beta3 subunit. These events with phosphorylation and dephosphorylation were clearly seen during reversible platelet aggregation with ADP. ILK possesses a pleckstrin homology domain which is involved in the regulation of its kinase activity through a PI3-kinase dependent pathway. Inhibition of PI3-kinase by two unrelated inhibitors (wortmannin and **LY294002**) prevented relocation of ILK in PMA-stimulated platelets, abolished ILK activation and its association with beta3. However, PI3-kinase inhibition did not prevent the conformational change in a alphaIIbbeta3 (checked through PAC-1 binding in flow cytometry), although it decreased the affinity of the integrin for fibrinogen as measured using FITC-fibrinogen. Furthermore aggregate formation was reduced. In summary, these results show that ILK transiently associates with and phosphorylates the beta3 subunit in a PI3-kinase dependent manner suggesting that ILK participates at an intermediate stage in a critical mechanism for assuring a stable and strong interaction with fibrinogen allowing stable aggregate formation.

L12 ANSWER 4 OF 5 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2001:193381 BIOSIS
DOCUMENT NUMBER: PREV200100193381
TITLE: Regulation of cell adhesion and neurite outgrowth by **integrin-linked kinase** in N1E-115 cells.
AUTHOR(S): Ishii, Toshiaki (1); Satoh, Eiki (1); Nishimura, Masakazu (1)
CORPORATE SOURCE: (1) Dept. Pharmacol., Univ. Obihiro Sch. Vet. Med., Obihiro, Hokkaido, 080-8555 Japan
SOURCE: Japanese Journal of Pharmacology, (2001) Vol. 85, No. Supplement 1, pp. 267P. print.
Meeting Info.: 74th Annual Meeting of the Japanese Pharmacological Society Yokohama, Japan March 21-23, 2001 Japanese Pharmacological Society
. ISSN: 0021-5198.
DOCUMENT TYPE: Conference
LANGUAGE: English
SUMMARY LANGUAGE: English

L12 ANSWER 5 OF 5 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 1999382019 MEDLINE
DOCUMENT NUMBER: 99382019 PubMed ID: 10454216
TITLE: Mechanism of protein kinase B activation by insulin/insulin-like growth factor-1 revealed by specific inhibitors of phosphoinositide 3-kinase--significance for diabetes and cancer.
AUTHOR: Galetic I; Andjelkovic M; Meier R; Brodbeck D; Park J; Hemmings B A
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SOURCE: PHARMACOLOGY AND THERAPEUTICS, (1999 May-Jun) 82 (2-3) 409-25. Ref: 198
Journal code: 7905840. ISSN: 0163-7258.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, ACADEMIC)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199910
ENTRY DATE: Entered STN: 20000111
Last Updated on STN: 20010730
Entered Medline: 19991026

AB Protein kinase B (PKB) is a member of the second messenger subfamily of protein kinases. The three isoforms of PKB identified have an amino-terminal pleckstrin homology domain, a central kinase domain, and a carboxy-terminal regulatory domain. PKB is the major downstream target of receptor tyrosine kinases that signal via the phosphoinositide (PI) 3-kinase. The crucial role of lipid second messengers in PKB activation has been dissected through the use of the PI 3-kinase-specific inhibitors wortmannin and **LY294002**. Receptor-activated PI 3-kinase synthesises the lipid second messenger PI-3,4,5-trisphosphate, leading to the recruitment of PKB to the membrane. Membrane attachment of PKB is mediated by its pleckstrin homology domain binding to PI-3,4,5-trisphosphate or PI-3,4-bisphosphate with high affinity. Activation of PKB alpha and beta is then achieved at the plasma membrane by phosphorylation of Thr308/309 in the A-loop of the kinase domain and Ser473/474 in the carboxy-terminal regulatory region, respectively. The upstream kinase that phosphorylates PKB on Thr308, termed PI-dependent protein kinase-1, has been identified and extensively characterised. A candidate for the Ser473/474 kinase, termed the **integrin-linked kinase**, has been identified recently. Activated PKB is implicated in glucose metabolism, transcriptional control, and in the regulation of apoptosis in many different cell types. Stimulation of PKB activity protects cells from apoptosis by phosphorylation and inactivation of the pro-apoptotic protein BAD. These results could explain why PKB is overexpressed in some ovarian, breast, and pancreatic carcinomas.

L13 ANSWER 1 OF 5 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1
ACCESSION NUMBER: 2002:451376 BIOSIS
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TITLE: Evidence that the platelet integrin alphaIIbbeta3 is regulated by the **Integrin-Linked Kinase**, ILK, in a PI3-kinase dependent pathway.
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L13 ANSWER 3 OF 5 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
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CORPORATE SOURCE: (1) Dept. Pharmacol., Univ. Obihiro Sch. Vet. Med., Obihiro, Hokkaido, 080-8555 Japan
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DOCUMENT NUMBER: 99382019 PubMed ID: 10454216
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CORPORATE SOURCE: Friedrich Miescher Institute, Basel, Switzerland.
SOURCE: PHARMACOLOGY AND THERAPEUTICS, (1999 May-Jun) 82 (2-3) 409-25. Ref: 198
Journal code: 7905840. ISSN: 0163-7258.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
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LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199910
ENTRY DATE: Entered STN: 20000111
Last Updated on STN: 20010730
Entered Medline: 19991026

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DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: MEDLINE; Priority Journals
OTHER SOURCE: MEDLINE 1998409636
ENTRY MONTH: 199810
ENTRY DATE: Entered STN: 19990122
Last Updated on STN: 20020726

AB **Integrin-linked kinase** (ILK) is an ankyrin-repeat containing serine-threonine protein kinase capable of interacting with the cytoplasmic domains of integrin beta1, beta2, and beta3 subunits. Overexpression of ILK in epithelial cells disrupts cell-extracellular matrix as well as cell-cell interactions, suppresses suspension-induced apoptosis (also called Anoikis), and stimulates anchorage-independent cell cycle progression. In addition, ILK induces nuclear translocation of beta-catenin, where the latter associates with a T cell factor/lymphocyte enhancer-binding factor 1 (TCF/LEF-1) to form an activated transcription factor. We now demonstrate that ILK activity is rapidly, but transiently, stimulated upon attachment of cells to fibronectin, as well as by insulin, in a phosphoinositide-3-OH kinase [Pi(3)K]-dependent manner. Furthermore, phosphatidylinositol(3,4,5)trispho sphate specifically stimulates the activity of ILK in vitro, and in addition, membrane targetted constitutively active Pi(3)K activates ILK in vivo. We also demonstrate here that ILK is an upstream effector of the Pi(3)K-dependent regulation of both protein kinase B (PKB/AKT) and glycogen synthase kinase 3 (GSK-3). Specifically, ILK can directly phosphorylate GSK-3 in vitro and when stably, or transiently, overexpressed in cells can inhibit GSK-3 activity, whereas the overexpression of kinase-deficient ILK enhances GSK-3 activity. In addition, kinase-active ILK can phosphorylate PKB/AKT on serine-473, whereas kinase-deficient ILK severely inhibits endogenous phosphorylation of PKB/AKT on serine-473, demonstrating that ILK is involved in agonist stimulated, Pi(3)K-dependent, PKB/AKT activation. ILK is thus a receptor-proximal effector for the Pi(3)K-dependent, extracellular matrix and growth factor mediated, activation of PKB/AKT, and inhibition of GSK-3.

USSN 08 1955841 Generate Collection

L12: Entry 1 of 2 File: USPT Jan 11, 2000

US-PAT-NO: 6013782
DOCUMENT-IDENTIFIER: US 6013782 A

TITLE: Integrin-linked kinase and its uses

DATE-ISSUED: January 11, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Dedhar; Shoukat	Vancouver			CA
Hannigan; Greg	Ontario			CA

US-CL-CURRENT: 536/23.1; 435/320.1, 435/325, 435/70.1

CLAIMS:

What is claimed is:

1. An isolated nucleic acid encoding a mammalian integrin-linked kinase (ILK) protein wherein said ILK protein has the amino acid sequence of SEQ ID NO:2.

2. An isolated nucleic acid wherein the nucleotide sequence of said nucleic acid comprises the sequence set forth in SEQ ID NO:1.

3. An isolated nucleic acid that will hybridize to the nucleotide sequence set forth in SEQ ID NO:1 under the following conditions: 0.1.times.SSC (9 mM saline/0.9 mM sodium citrate) at 50.degree. C. or higher wherein said nucleic acid encodes a human ILK protein.

4. An expression cassette comprising a transcriptional initiation region functional in an expression host, a nucleic acid having a sequence of the isolated nucleic acid according to claim 1 under the transcriptional regulation of said transcriptional initiation region, and a transcriptional termination region functional in said expression host.

5. An isolated cell comprising an expression cassette according to claim 4 as part of an extrachromosomal element or integrated into the genome of a host cell as a result of introduction of said expression cassette into said host cell, and the cellular progeny of said host cell.

6. A method for producing mammalian ILK protein, said method comprising: growing a cell according to claim 5, whereby said mammalian ILK protein is expressed; and isolating said ILK protein free of other proteins.

USSN 09/566906 ☐ Generate Collection

L6: Entry 7 of 9 File: USPT Apr 9, 2002

US-PAT-NO: 6369205
DOCUMENT-IDENTIFIER: US 6369205 B1

TITLE: Integrin-linked kinase and its uses

DATE-ISSUED: April 9, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Dedhar; Shoukat	Vancouver			CA
Hannigan; Greg	Ontario			CA

US-CL-CURRENT: 530/388.26; 424/139.1, 424/142.1, 530/387.1, 530/388.1, 530/388.15

CLAIMS:

What is claimed is:

1. An antibody that specifically binds to a human integrin-linked kinase (ILK) polypeptide encoded by SEQ ID NO:1.

2. The antibody of claim 1, wherein said antibody is a monoclonal antibody.

3. The monoclonal antibody of claim 2, wherein said antibody blocks binding of ILK to integrin.

4. The monoclonal antibody of claim 2, wherein said antibody binds to the ILK kinase domain.

5. The monoclonal antibody of claim 2, wherein said antibody binds to an ankyrin-like repeat present on said human integrin linked kinase.

End of Result Set 09/035706 USSN

Generate Collection

L6: Entry 9 of 9 File: USPT Dec 14, 1999

US-PAT-NO: 6001622
DOCUMENT-IDENTIFIER: US 6001622 A

TITLE: Integrin-linked kinase and its use

DATE-ISSUED: December 14, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Dedhar; Shoukat	Vancouver			CA
Hannigan; Greg	Ontario			CA

US-CL-CURRENT: 435/194; 435/15

CLAIMS:

What is claimed is:

1. A method of inhibiting the catalytic activity of mammalian integrin linked kinase (ILK) in a cell, the method comprising:

decreasing the available level of {Ptdlns (3,4,5) P.sub.3 } in said cell by administering to said cell an agent selected from the group consisting of analogs of {Ptdlns(3,4,5)P.sub.3 }; mimetics of the ILK {Ptdlns(3,4,5)P.sub.3 } binding site and wortmannin; at a concentration effective to inhibit the catalytic activity of said mammalian ILK.

2. A method according to claim 1, wherein said binding of {Ptdlns (3,4,5) P.sub.3 } to ILK occurs at one or both of the amino acid residues; lys162 and lys209.

3. A method according to claim 1, wherein said inhibition of ILK results in decreased phosphorylation of protein kinase B at amino acid residue ser473.

4. A method according to claim 1, wherein said inhibition of ILK results in increased activity of glycogen synthase kinase 3.

☐ Generate Collection

USSN 09/390425

L6: Entry 8 of 9 File: USPT Jan 15, 2002

US-PAT-NO: 6338958
DOCUMENT-IDENTIFIER: US 6338958 B1

TITLE: Integrin-linked kinase and its uses

DATE-ISSUED: January 15, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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CLAIMS:

What is claimed is:

1. A method of in vitro screening for biologically active agents that modulate the phosphorylation activity of integrin linked kinase (ILK), the method comprising:

combining in a reaction mixture a candidate biologically active agent; a human ILK polypeptide; a substrate for ILK kinase; and ATP;

measuring the phosphorylation of said substrate in the presence of said candidate biologically active agent, compared to a control sample in the absence of said agent;

wherein a decrease in phosphorylation is indicative that said agent is an inhibitor of ILK and an increase in phosphorylation is indicative that said agent is an enhancer of ILK.

2. A method of claim 1, wherein said reaction mixture further comprises (Ptdlns (3,4,5)P.sub.3) and wherein said agent inhibits the binding of (Ptdlns (3,4,5)P.sub.3) to ILK.

3. The method of claim 1, wherein said ILK substrate is protein kinase B at amino acid residue ser473.

4. The method of claim 1, wherein said substrate is ILK.